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The Seroprevalence Rates and Approach Management of Brucellosis in Thailand

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ABSTRACT

Background: Brucellosis is an important re-emerging zoonosis with worldwide distribution. In Thailand, endemic bovine and caprine brucellosis is a serious public health threat. Brucellosis in cattle and buffalo is primarily due to *Brucella abortus* while *Brucella melitensis* predominates in sheep and goats. The main factors maintaining the disease in humans and animals are unhygienic food habits, poor animal husbandry processes, lack of awareness and ineffective surveillance systems. Therefore, prevention of human brucellosis would depend on controlling the disease in livestock. The current study aimed to study the epidemiological situation to develop an effective control strategy.

Methods: The estimated seropositivity rates at the herd and animal levels were compiled during 2009-2018. The sera were submitted to the National Institute of Animal Health (NIAH) and the Regional Veterinary Research and Development Centers (VRDCs), where serological tests were performed. The testing used screening based on the Rose Bengal test (RBT) followed by confirmatory tests, the complement fixation test (CFT) and the indirect enzyme-linked immunosorbent assay (I-ELISA). The data were collected, the proportion of the estimated seropositivity rate was analyzed. The seropositive animals were followed up and specimens were collected for isolation and PCR identification.

Result: The results of the accumulated seropositivity rate of the brucellosis of dairy cattle, beef cattle, buffaloes, goats and sheep at the herd level from 2009 to 2018 was 7.12% (95% CI: 6.99-7.26) and the animal level was 0.92% (95% CI: 0.91-0.93). The species identification presented *Brucella abortus* five isolates, whereas *Brucella melitensis* presented 31 isolates from the total of 36 isolates. This information of the serological status and *Brucella* spp., including diagnostics patterns could be used as base data for better managing the prevention and control measures of brucellosis in Thailand and other countries.

Key words: Brucellosis, Management, Seroprevalence.

INTRODUCTION

Brucellosis, caused by organisms of the genus *Brucella*, is a major, worldwide zoonosis with significant economic importance in agriculture and public health. In cattle, sheep and goat brucellosis is a highly contagious condition manifested clinically by abortions and infertility. Humans are not a source of contagion for brucellosis; domestic animals constitute the main reservoir for human infection (Kumar *et al*., 2010; Moreno, 2014). *Brucella*, a Gram-negative bacillus, is a member of the *Brucellaceae* family in the order Rhizobiales and class Alphaproteobacteria. The genus currently consists of 12 recognized species (WOAH, 2022), is highly homogenous with identical 16S rRNA (Gee *et al*., 2004) and recA gene sequences (Scholz *et al*., 2008) and displays more than 95% of homology based on DNA-DNA hybridization studies (Verger *et al*., 1985). Three species of major public health concern and economic importance are *B. abortus* which affects cattle, *B. melitensis* which predominantly infects sheep and goats and *B. suis* which affects swine. Brucellosis was first reported in Thailand in 1956 (Visudhthiphan and Na-Nakorn, 1970) and has since spread throughout the country as endemic. Brucellosis is a notifiable disease according to Thailand's Animal Epidemics Act B.E. 2499 (1956) and its revision B.E. 2558 (2015). In Thailand, endemic bovine and caprine brucellosis could cause serious public health threats (Laosiritaworn *et al*., 2007; Lapphral *et al*., 2014). Brucellosis in cattle and buffalo is also primarily due to *B. abortus*, while *B. melitensis* predominates in sheep and goats. An increase in recent cases of human brucellosis has thus caused alarm (Laosiritaworn *et al*., 2007; Wongphruksasoong, 2010). In additional, the Department of Livestock Development (DLD) announced the National Brucellosis Control and Eradication Program (NBCEP) to minimize the economic impact of the disease on livestock and reduce the public health risks. Furthermore, brucellosis surveillance activities in the laboratory have been conducted regularly by the DLD. The diagnosis of brucellosis is based on the field condition of that area, history of the animals and relies on the serological results for the serodiagnosis of brucellosis, including the

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Rose Bengal test (RBT), the complement fixation test (CFT), the fluorescence polarization assay (FPA) and the indirect enzyme-linked immunosorbent assay (I-ELISA). Never theless, no single serological test is appropriate in all epidemiological situations and for all animal species; moreover, all tests have limitations, especially when screening individual animals. Therefore, consideration should be given to all factors that impact the relevance of the test method and results to a specific diagnostic interpretation or application. Hence, the brucellosis situation is needed to be described for the based data of the country for further control and elimination of brucellosis in Thailand.

MATERIALS AND METHODS

This study was approved by the Animal Care and Use for the Scientific Research Committee, Kasetsart University, Thailand (Approval number ACKU62-VER-009). The number of the collected sera were designed under the Bureau of Disease Control and Veterinary Services (BDCVS), DLD for disease surveillance. The sera were collected by the field officers and submitted to the National Institute of Animal Health (NIAH) and the Regional Veterinary Research and Development Centers (VRDCs), where serological tests were performed. The total number of samples for testing were 140,287 herds with 3,187,323 serum samples during 2009-2018. The testing used screening based on the RBT followed by confirmatory tests, the CFT and the I-ELISA. The seropositive rates at the herd and animal levels were analyzed as the proportion of the estimated seropositivity rate of laboratory surveillance. The seropositive animals were slaughtered and specimens such as specified lymph nodes, liver and spleen were collected for isolation and molecular identification by PCR.

Identification of *Brucella*

Bacteriological isolation

The samples were collected using the following techniques: The samples were trimmed to remove the unnecessary fat before chopping and resuspending in sterile phosphate-buffered saline (PBS). Then, one-two drops were streaked onto a solid medium base supplemented with 5% of horse serum and onto Farrell's medium and selective medium. After incubation of three-five days, the suspected colonies were examined for the growth of *Brucella* spp. Suspected *Brucella* colonies were characterized using their round, pinpoint and clear yellow appearance and examined for gram staining and modified Ziehl-Neelsen stain (MZN). Subsequent biochemical tests were carried out for oxidase, catalase, urease production, methyl red and acid production on media containing the suspected colonies, including growth on dyes and agglutination with monospecific sera.

Genus-specific PCR: Multiplex PCR (Bruce-ladder)

Genomic DNA was extracted from the isolates using a QIAamp DNA Mini Kit (Qiagen, USA). The multiplex PCR (Bruce-ladder) using eight primers could differentiate most *Brucella* species, including marine mammals and the vaccine strains of *B. abortus* S19, *B. abortus* RB51 and *B. melitensis* Rev.1 in a single reaction (Kaur *et al*., 2018; López-Goñi *et al*., 2008). The Bruce-ladder PCR was defined as 0.32 pmol/µL of each primer, 0.05 U Takara Ex Ta® (Takara BIO INC, Japan), 0.25 mmol -dNTP and 1X Takara Ex buffer (Takara BIO INC, Japan) to a final volume of 25 µL with 3 µL of this DNA at 10-100 ng /µL. The reaction went through a period of denaturation at a temperature of 95°C for 3 min, followed by 35 cycles at a temperature of 95°C for 75 s, 55.5°C for 80 s, 72°C for 80 s and a final extension cycle at 72°C for 5 min. To confirm the PCR results, the products were visualized on 1.5% of agarose gel electrophoresis with a 45 min running time at 100 V. The results were visualized by the non-toxic dyes, such as SYBR® green. The intensity of the band could be used to estimate the amount of the product of given molecular weight relative to a ladder.

RESULTS AND DISCUSSION

The number of the tested herds and animals over the country during 2009-2018 were shown in Table 1. The results of the accumulated seropositivity rates of the brucellosis of dairy

Table 1: Number of tested herds and animals for brucellosis during 2009-2018.

Year	Dairy		Beef		Buffalo		Goats		Sheep		Total	
	Herds	Animals	Herds	Animals	Herds	Animals	Herds	Animals	Herds	Animals	Herds	Animals
2009	2652	60452	2885	43397	321	5957	2508	78254	128	3326	8494	191386
2010	4018	74457	3424	37308	340	5742	2710	76320	225	5612	10717	199439
2011	4274	88741	3591	34880	311	4971	3083	105849	190	5174	11449	239615
2012	12325	258401	2243	41691	653	7882	4273	151800	316	8122	19810	467896
2013	7114	182998	2024	36544	362	6633	5221	119594	313	9850	15034	355619
2014	4921	131437	2534	36971	1012	14911	5001	170056	374	11954	13842	365329
2015	5798	121241	2722	43640	2499	24536	5221	68176	428	12579	16512	361160
2016	3950	69230	1736	41290	1656	20675	4831	148217	367	10213	12540	289625
2017	4061	82185	4253	63529	1441	18792	5101	172775	367	11409	15223	348690
2018	3670	102451	3057	58858	2688	23393	6725	169588	526	14274	16666	368564
Total	52783	1171593	28469	438108	11283	133492	44674	1260629	3234	92513	140287	3187323

cattle, beef cattle, buffaloes, goats and sheep at the herd level was 7.12% (95% CI: 6.99-7.26) and the animal level was 0.92% (95% CI: 0.91-0.93). Dairy cattle at the herd level were 3.76% (95% CI: 3.60-3.92) (Fig 1) and 0.37% (95% CI: 0.36-0.38) at the animal level (Fig 2). After 2012, the testing policies and slaughtering were performed conscientiously and by 2013 the percentage of positive results at the herd level had decreased to 2%. However, the number of the tested herd and animals decreased because the focus was on infected herds and the percentage of the positive results remained high at about 5%. The percentage of the positive results at the herd level remained consistent from 2015 to 2018, with a 2% positive percentage at the herd level. The seropositivity rate in beef cattle was 9.17% (95% CI: 8.83-9.50) at the herd level (Fig 3) and 2.07% (95% CI: 2.02-2.19) at the animal level (Fig 4), where as in buffaloes at the herd level, it was 3.45% (95% CI: 3.12-3.79) and at the animal level 0.72% (95% CI: 0.68-0.77). In goats, it was 9.65% (95% CI: 9.38-9.92) at the herd level (Fig 5) and 1.89% (95% CI: 1.87-1.91) at the animal level (Fig 6). The highest percentage of the seropositivity rates in goats at 15% was recorded from 2011 to 2012. After 2012, the national program for brucellosis control focused on goat herds and by 2015, the seropositive percentage at the herd level had decreased to 6.2%. However, from 2015 to 2018, the seropositivity rate at the herd level increased but decreased at the individual level. During this period, the DLD encouraged livestock farmers to raise small ruminants and the number of herds increased. While sheep herds had the highest seropositive results, it was 17.81% (95% CI: 16.49-19.12) at the herd level and 3.08% (95% CI: 2.98-3.18) at the animal level. The number of sheep herds in Thailand was also lower than for other livestock such as cattle or goats. Sheep farming mostly involved ecotourism, so if the sheep did not appear to have any serious problems, the owners did not worry about animal health. Nonetheless, the trends for infected herds and animals decreased.

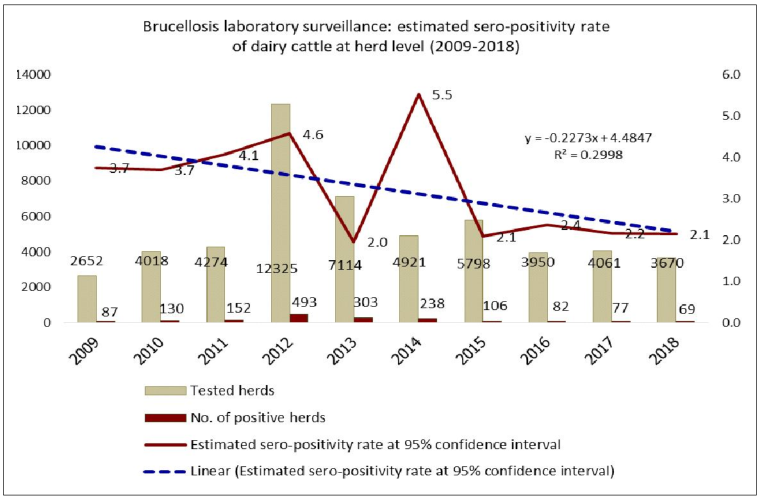

Fig 1: Herd level seropositivity rate of brucellosis in dairy cattle from 2009 to 2018.

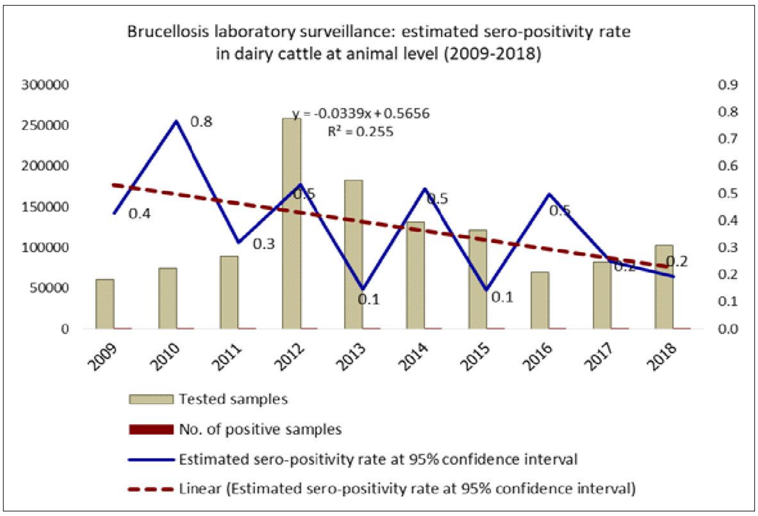

Fig 2: Animal level seropositivity rate of brucellosis in dairy cattle from 2009 to 2018.

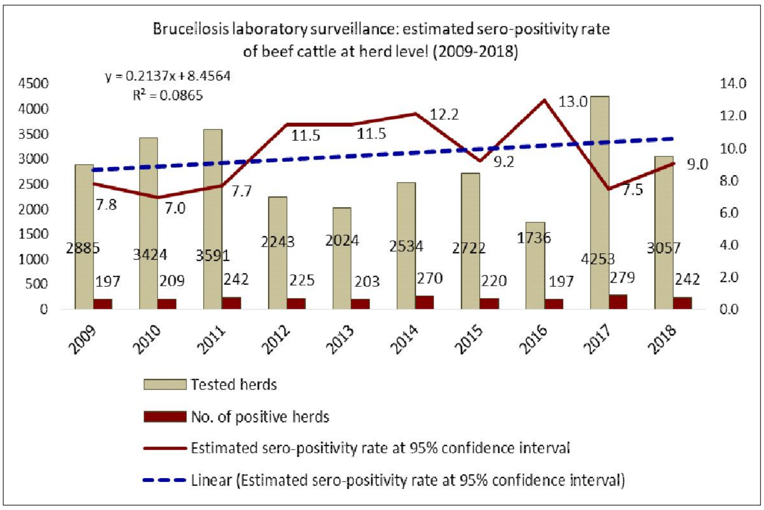

Fig 3: Herd level seropositivity rate of brucellosis in beef cattle from 2009 to 2018.

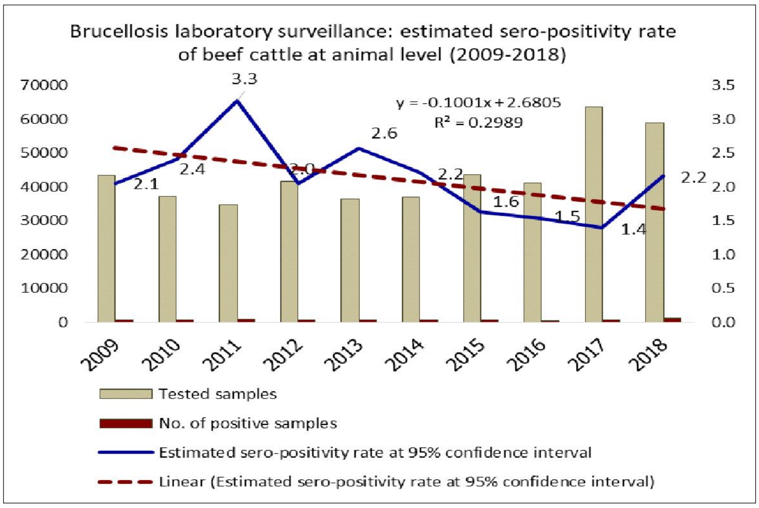

Fig 4: Animal level seropositivity rate of brucellosis in beef cattle from 2009 to 2018.

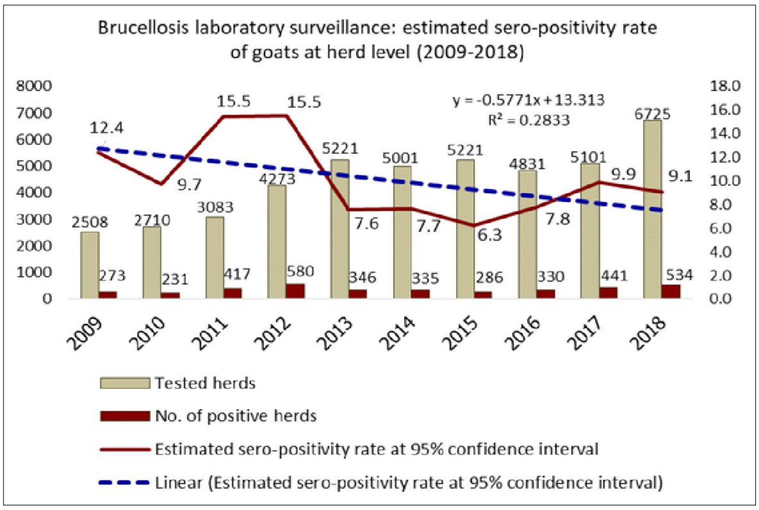

Fig 5: Herd level seropositivity rate of brucellosis in goats from 2009 to 2018.

The previous data reviewed the prevalence in the most numbers of livestock in Southeast Asia. The prevalence of bovine brucellosis (*Brucella abortus*) in 2014 ranged between 1-2% in Thailand and Indonesia and 4-5% in Malaysia and Myanmar. Additionally, the prevalence of goat brucellosis (*Brucella melitensis*) is approximately 1% in Malaysia and Thailand (Zamri-Saad and Kamarudin, 2016). *Brucella* isolation and identification showed five *Brucella abortus* isolates, whereas there were 31 *Brucella melitensis* isolates from the total of 36 isolates. However, a definitive diagnosis would require the isolation and identification of the *Brucella* organism, but this method is impractical for large-scale testing and has poor sensitivity depending on the host species and duration of infection. In addition, not all infected animals can be tracked for specimen collection.

Recommendations

The 'test-and-slaughter' method is recommended and feasible in countries where prevalence rate is not exceeding 2% (Alton, 1987). At the herd prevalence (7.12% over all species), the 'test-and-slaughter' seemed unsuitable for Thailand. Nevertheless, the national brucellosis control program in Thailand had successfully reduced the prevalence. However, the disease elimination is quite difficult as well as human cases still be reported. Therefore, the implementation of brucellosis control policy in Thailand needs to be reviewed.

These recommendations may focus on some weaknesses to get closer to the goal of eliminating the disease in Thailand.

Surveillance system

The national list agreed by the DLD is based on the WOAH listed diseases and the Animal Epidemics Act 2015. This list is regularly reviewed and updated according to the WOAH -listed diseases and national concerns. The diseases are included for surveillance purposes, so that unusual incidences of animal morbidity or mortality and diseases of public health significance can be quickly detected. The requirement to report notifiable diseases is enforced by law. Surveillance is required for domestic animals in any particular area and also for animal movement control and transport to slaughterhouses. Any positively infected cattle must be culled, with appropriate compensation in terms of price and reasonable payment time. Brucellosis screening tests must be improved to detect infected animals as quickly as possible. An epidemiological investigation should be conducted to identify the risk factors associated with the occurrence and endemicity of the disease.

Increasing certification of brucellosis-free farms

Registered brucellosis-free farms are allowed to move their animals freely, without movement testing. Therefore, increasing the amount of free farm testing is a crucial control measure to better determine the true prevalence of brucellosis among animal populations. Increased testing could be encouraged by offering incentives, increasing the amount of free farm testing during trading or increasing disease awareness among farmers or disease-affected populations. The DLD has a standard operating protocol for farmers on how to register as a brucellosis-free farm.

Educating and encouraging farmers

Educating farmers in disease introduction, relevant regulations and adjusting their attitudes are important initiatives. Farmers should be encouraged to learn more about disease prevention and control. The DLD and Public Health Departments have produced pamphlets to educate farmers about diseases, contact, symptoms and prevention and the procedure to follow when suspecting disease occurrence. Animal identification (ID), moving control, good farm practice and screening animals before purchasing must be permanent practices routinely applied on their farms. *Brucella* is mainly found in the uterus and placenta and spread by secretion during delivery. Thus, to reduce the spread of the disease, delivery management such as separating an animal in labor from other animals during

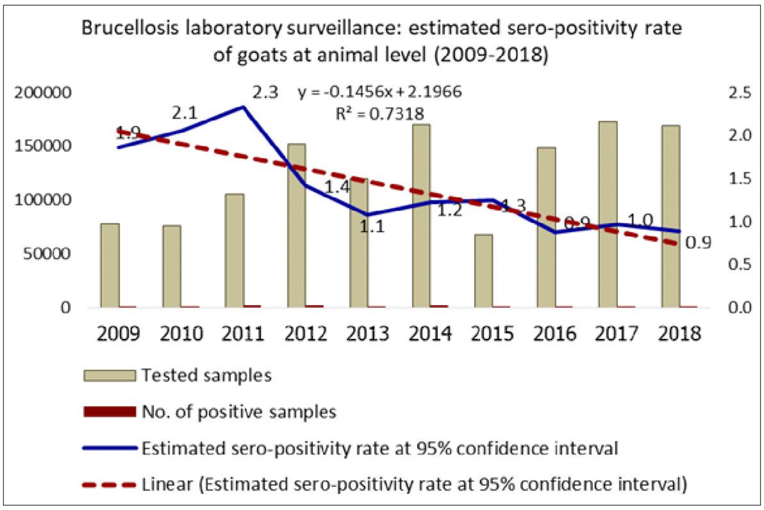

Fig 6: Animal level seropositivity rate of brucellosis in goats from 2009 to 2018.

parturition is necessary to reduce the transmission of the agent across the herd.

One health approach

The one health concept recognizes that the health of people is connected to the health of animals and the environment and involves cooperation among physicians, veterinarians and ecologists. One health is defined as a collaborative, multisectoral and transdisciplinary approach at local, regional, national and global levels with the goal of achieving optimal health outcomes and recognizing the interconnection between people, animals and plants and their shared environment. Human brucellosis investigation is an effective method to provide collaboration among animals. However, the clinical signs of human brucellosis are atypical, including a mostly undulant fever that can cause misdiagnosis among physicians and lead to underreported case numbers. To resolve these problems, an information exchange mechanism between the human and animal sectors should be established and enhanced.

Increasing vaccination efficacy

In Thailand*, B. abortus* S19 strain vaccination remains the only permitted vaccine that is given to female calves aged between 3 and 6 months in high-risk areas via a single subcutaneous dose of 5-8$\times10^{10}$ viable organisms. The test-and-slaughter policy is still be set with problems such as compensation, animal ID or specific guidelines. Vaccination is a valuable method in the effort to decrease disease prevalence. However, vaccination policy implementation in Thailand should be reconsidered by reducing the dose from 3×10^{8} to 3×10^{9} organisms that can be administered subcutaneously to adult cattle. The differences in immune responsiveness after immunization in cattle of different genotypes, which should be tested in all breed in Thailand or at least the basic breeds (Kumar, 2018).

Meat inspection

Government compensation for slaughtered animals is only 75% of the market price and the process usually takes a long time, resulting in farmers lacking the incentive to control the disease more effectively. Meat from slaughtered animals could be consumed according to FAO guidelines (Herenda and Chambers, 2000). The FAO has developed guidelines for meat inspection in developing countries, where the aim of meat inspection is to provide safe and wholesome products for human consumption. In many developing regions, meat inspectors often lack the necessary guidelines to assess the sanitary status of carcasses, meat and organs from slaughtered animals. Brucellosis is one of the diseases mentioned in the guidelines. *Brucella* organisms have only a short lifespan in the muscles after slaughter and this impacts the safety of skeletal muscle meat. Cattle and horse carcasses affected with brucellosis are approved for consumption after removal of the affected parts. In the acute abortive form after a miscarriage, the cattle carcasses are condemned. Pig, sheep, goat and buffalo carcasses require total condemnation. Affected parts of the carcass, such as the udder, genital organs and corresponding lymph nodes must be condemned. In Thailand, the Control of Animal Slaughter for the Distribution of Meat Act B.E.2559 (2016) does not allow the distribution of meat from infected animals. This issue should be considered nationally and the law should be amended to be appropriate and in accordance with the FAO guidelines.

CONCLUSION

Brucellosis remains a serious threat to humans and animals in Thailand. The current surveillance capabilities would provide a better understanding of the epidemiological situation so to develop an effective control strategy. Proper applications of testing and slaughter procedures, accompanied by vaccination and a strict surveillance scheme, would be the first steps to reduce infection pressure and limit the spread of the disease. Following suitable meat inspection, meat from slaughtered animals could be consumed following the Food and Agriculture Organization (FAO)'s guidelines. Additionally, the capacity of veterinary services, public awareness and intersectoral collaboration are key components of brucellosis control. Likewise, monitoring and evaluating the testing results would be essential control measures to successfully reduce each parameter of disease incidence.

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Conflict of interest

The authors declare that they have no conflict of interest.

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